K562/GM-CSF VACCINATION IN COMBINATION WITH IMATINIB MESYLATE (GLEEVEC™) FOR CHRONIC MYELOID LEUKEMIA (CML)

Nontechnical Abstract

Chronic Myelogenous Leukemia is a cancer of the bone marrow cells that are the precursors of all cellular elements of the blood. It accounts for roughly 20% of all adult leukemias. The disease typically presents in a "chronic phase" in which there is an increased number of cells in the marrow, an elevated number of white blood cells and platelets in the blood, and often an enlarged spleen. This phase may be asymptomatic, or symptoms may relate to the enlarged spleen. The chronic phase of the disease typically lasts from 3-6 years, after which an "accelerated phase" of the disease arises, usually symptomatic and requiring increasing doses of medications to control the rising white blood cell counts. This is invariably followed by "blast crisis", which is often refractory to standard therapies and is lethal.

The initiating cause(s) of CML is/are largely unknown, although a great deal is known about the molecular biology of this disease. The genetic hallmark of CML is a rearrangement of part of chromosome 9 with that of chromosome 22 forming a "hybrid" known as the Philadelphia (Ph) chromosome which is present in virtually every leukemic cell. The reshuffling of the DNA from these two chromosomes results in regions of a gene known as *BCR* (chromosome 22) fusing with regions of a gene known as *ABL* (chromosome 9) to form the hybrid *BCR-ABL* fusion gene. This fused gene and its protein product are uniquely expressed in the leukemia cell, and are not found in any normal cell in the body. The protein made from the fusion gene contains a critical region that promotes cell growth and survival, and regulation of its function is lost with the fusion protein, leading to the transformation of blood forming cells and the clinical features described above

Standard treatment of CML includes the following:

Hydroxyurea and busulfan

Historically, these oral drugs have been the standard therapy for chronic phase CML. While these treatments are usually successful in controlling the blood counts, they do not generally result in eliminating cells with the Philadelphia Chromosome and do not alter the transformation of CML into terminal blast crisis.

Interferon-α

In chronic phase CML, interferon-α can induce complete cytogenetic responses (elimination of all detectable Philadelphia chomosome containing cells in the blood and marrow) in 5 to 20% of patients and prolong survival. It specifically affects proliferation and differentiation of the leukemic clone and augments immune responses against CML-associated antigens. However, patients who attain cytogenetic responses on interferon-α typically harbor persistent molecular evidence of BCR-ABL. Furthermore, side effects of interferon can be intolerable and limit long-term treatment in a substantial number of patients.

Imatinib mesylate

In addition to interferon, imatinib mesylate is now widely considered to be front-line therapy for CML. Imatinib is a potent inhibitor of the protein tyrosine kinase of the BCR-ABL fusion protein that leads to uncontrolled cell growth.

The initial phase I clinical trials of imatinib revealed dramatic responses in CML patients who failed interferon–α, with 53 of 54 patients normalizing their white blood cell (WBC) count and platelet counts, usually within four weeks of initiating therapy. In this study, 54% of patients had cytogenetic responses (31% major and 13% complete), and these were demonstrable significantly earlier than with interferon. Several subsequent multi-institutional phase II trials have been reported involving more than 1000 patients in chronic or accelerated phase CML. More than 90% of patients with interferon resistant chronic phase disease normalized their blood counts with imatinib, and nearly half had a major cytogenetic response, with complete cytogenetic responses obtained in over 40%. In the IRIS study, at a median 19 month follow-up, 95% of

patients treated initially with imatinib had a complete hematologic response, and 85% had a major cytogenetic response, most of which were complete.

Despite the promising results with imatinib, resistance to imatinib can occur. Some patients treated in chronic phase who initially respond to imatinib subsequently relapse with highly aggressive resistant disease. Recent reports have suggested that the primitive Ph chromosome positive CML stem cells (the "seeds" of the leukemia) are insensitive to imatinib *in vitro*, such that imatinib alone may be incapable of truly eradicating the disease. Furthermore, several studies examining molecular detection of BCR-ABL by a test known as RT-PCR that is able to detect as few as one in a million leukemia cells, have reported that most cytogenetic responses to imatinib, including complete responses, remain BCR/ABL positive.

In one study, while all cytogenetic responders on imatinib had a measurable decrease in tumor burden by RQ-PCR, only 2 of 13 patients reached residual disease below 10^{-2}. All responding patients reached a plateau by four months of therapy, and no patient reached BCR/ABL negativity. In an interim analysis of the IRIS study, only 2 of 28 patients receiving imatinib as first-line therapy reached PCR-undetectable transcript levels, and the rate of response slowed as a function of duration of therapy. Finally, of 21 patients who achieved a complete cytogenetic remission on imatinib with very low or RT-PCR-undetectable transcript levels, two-thirds had subsequent increases in tumor burden, with 19% having cytogenetic relapse, 27% having a sustained > 1 log increase in BCR/ABL transcripts, and 33% converting from undetectable to detectable disease status.

Allogeneic bone marrow transplantation

Chronic phase CML was among the first diseases shown to be curable by transplantation of bone marrow cells from a related donor (allogeneic BMT). Survival rates of 50 to 60 percent have been reported among young patients with CML in chronic phase who received such a transplant, and long-term follow-up strongly suggests these patients are cured.

Whereas allogeneic BMT has been a longstanding treatment of choice for eligible patients, a significant fraction die from transplant-related complications, and over two-thirds of patients either do not have a suitable donor or are considered too old to safely tolerate transplantation. Recent experience with less intensive transplants and alternate donor sources have attempted to overcome these limitations, although at present, these experimental therapies still carry a significant risk of complications.

The transplant experience in CML has provided the most compelling evidence that CML is an "immunologically responsive disease". Specifically, T lymphocytes from allogeneic donors have been shown to be highly potent at eradicating the leukemia, whether these T cells are present in the original marrow (or peripheral blood) "graft" or given later as an infusion of donor lymphocytes.

Rationale for Integrating a CML "Cancer Vaccine" with Imatinib.

Because of the remarkable ability of imatinib to reduce the number of CML cells to almost undetectable levels with minimal toxicity, many oncologists have moved this drug up as "front-line" therapy for CML. Patients treated on this drug respond quickly (within weeks), and generally feel well. Importantly, they remain immunologically healthy, as imatinib is not toxic to normal cells of the immune system, and may even enhance immunity. Unfortunately, most patients treated with imatinib continue to have molecular evidence of the disease, as measured by highly sensitive tests that identify the rearranged bcr/abl gene in residual leukemia cells in the blood and marrow. These tests are remarkably quantitative, enabling the serial measurement of the amount of leukemia cells over time. Recent studies are emerging reporting patients who had initially responded to imatinib developing progressive increases in the number of bcr/abl positive cells, and this is often followed by frank clinical progression of the leukemia (relapse) that has become resistant to imatinib. Accordingly, a non-toxic therapy that could be added to the treatment of patients who have partially responded to imatinib is desirable.

Patients in this study will receive an experimental vaccine (K562/GM-CSF) in an attempt to generate and amplify immunity to the leukemia cells. K562 is a cell line that originated from a patient in the blast crisis phase of CML. This cell line has been shown to have the BCR/ABL translocation, and also expresses many of the same proteins found in the leukemia cells of most patients with CML. In this vaccine, K562 cells were altered by the introduction of a gene that leads to the producion of a protein known as GM-CSF, which is normally made by cells of the immune system to initiate immune responses. K562/GM-CSF cells have been irradiated so that they cannot grow. Mouse models and early phase clinical trials have shown that this type of cellular vaccine is capable of inducing strong immunity to cancer cells, and some clinical responses to this type of vaccine has been reported in patients with other types of cancer.

The primary goals of this study are to see if CML patients who have been taking imatinib for greater than one year, and have had a good reduction in the amount of leukemia cells but still have detectable disease, can respond to vaccination with K562/GM-CSF by mounting an immune response that can further reduce, or perhaps eliminate the residual leukemia cells. By virtue of having laboratory assays that can measure the frequency of T cells specific for certain defined CML associated protein antigens, we plan to test if vaccination with K562/GM-CSF increases the number of such T cells, and if so, whether this is associated with a measureable reduction or elimination of BCR/ABL positive cells in the blood.